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Acute and Subchronic Inhalation Exposures of Hamsters to Nickel-Enriched Fly Ash

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16. ABSTRACT
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Acute and Subchronic Inhalation Exposures of Hamsters to Nickel-Enriched Fly Ash

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One 6-hr inhalation exposure of hamsters to Ni-enriched fly ash (NEFA) aerosol (respirable aerosol concentration ~200 μg/liter) deposited about 80 μg in the deep lung, of which 75 μg was still present 30 days postexposure. The animals tolerated the exposure well during the 30-day postexposure observation period.

Two-month exposures of hamsters to NEFA or fly ash (FA) aerosols (~185 μg/liter) resulted in a deep lung burden of about 5.7 mg, dark discoloration of lungs, heavily dust-laden macrophages, and significantly higher lung weights than in controls, but only minimal inflammatory reaction and no deaths. There was no difference between NEFA and FA effects. The NEFA contained 9% Ni; FA contained 0.03% NI.

The results of this study indicate low acute and subchronic toxicity and slow lung clearance of NEFA and FA.

INTRODUCTION

Based on estimates by the Environmental Protection Agency (1973), close to 6 million tons of fly ash are being dispersed annually into the atmosphere from the combustion of coal in the United States (U.S. Bureau of Mines, 1970). Fly ash must thus be considered a major air pollutant to which appreciable parts of the population are being chronically exposed.

One of the trace elements in fly ash is nickel which, at least in some forms, has been found to be carcinogenic and/or otherwise toxic or pathogenic (National Academy of Sciences, 1975; National Institute for Occupational Safety and Health, 1977). Elemental analyses of ash from 827 U.S. coals by the Bureau of Mines indicated average values of 0.005% nickel (50 ppm) for ash from Western coals to nearly 0.03% nickel (300 ppm) for ash from Midwestern and Eastern coals (Abernethy *et al.*, 1969). Therefore, the apparent emission factor is in the approximate range from 50 to 300 tons of nickel per 100 million tons of coal. Natusch *et al.* (1974) have shown that the nickel content of coal fly ash increases with the reciprocal of particle diameter. The mean nickel content of particles increased fourfold as particle diameters decreased from 10 to 1 μm. Thus, concentrations of nickel in respirable particles are appreciably greater than those found in gross fly ash. Hayes *et al.* (1978) found that trace elements are not homogeneously distributed even in particles of similar size and that the mean content of a given trace element in fly ash is determined by relatively few particles with a very high content of that element. This means that cells in the respiratory tract or the lung would not come into contact with fly ash particles containing 0.03% nickel but with particles containing many times that quantity.

Comprehensive data on nickel in ambient air to which the public at large is

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exposed are published in the report of the Environmental Protection Agency on atmospheric nickel for 1968 and 1969 (EPA, 1973). Nickel concentrations in urban areas were markedly higher than in nonurban areas where they were mostly below detection limits. Large northern cities tended to have higher nickel concentrations than other cities. New York City ranked first in 1969 with a yearly average of 0.17 μg nickel/m^3; the mean concentration during the first (winter) quarter of that year was 0.33 μg/m^3. Other northern cities show similar seasonal variation. Corresponding to seasonal changes in fossil fuel combustion, which is the main source of nickel-polluted air to which the general population is exposed, southern cities had lower nickel concentrations and showed much less seasonal variation.

While nickel concentrations in ambient urban air are low, human exposures occur for lifetimes. U.S. energy consumption has been increasing more than 4% per year since 1960, and nearly 5% per year during 1972 and 1973 (U.S. Department of the Interior, 1974). Since recent experience shows national economic health dependent on energy availability, increased coal use is widely advocated to decrease anticipated energy shortages and dependence on imported fuel. Since fossil fuel combustion evidently is the major source of atmospheric nickel, its concentrations in the air, and with it human exposure to nickel in metropolitan air, will increase.

If a bioassay of fly ash does indicate detrimental effects, the question would arise whether these effects might, at least in part, be due to its nickel content rather than the fly ash per se. The aforementioned inverse relationship between nickel content and particle diameter and the findings of Hayes *et al.* lend more weight to this possibility. To investigate this question, we exposed groups of hamsters to fly ash (FA) or to nickel-enriched fly ash (NEFA) under otherwise identical conditions. Enrichment of FA with nickel also facilitated determination of the quantity of NEFA retained in the lungs of the exposed animals as a function of aerosol concentration and exposure time.

MATERIALS AND METHODS

Iron-rich Ohio coal from the Pittsburgh No. 8 seam was fired in a multifuel furnace at Battelle Columbus Laboratories and FA collected in a baghouse. The nickel content of the fly ash was increased from approximately 0.03% in FA to 9% in NEFA by adding 1 part by weight nickel acetate to 14 parts of pulverized coal prior to combustion. The chemical form in which nickel is present in FA and in NEFA is not known at this time.

The Syrian golden hamster (*Mesocricetus auratus*, outbred LAK:LVG, Charles River Laboratories) was chosen as the animal model because the positive response of this species to various pathogenic agents, including carcinogens, has been reported by a number of investigators (Wehner *et al.*, 1975; 1978).

The aerosol exposure system, shown in Fig. 1, has been previously described (Wehner *et al.*, 1972).

Acute Toxicity Test

The objective of the acute toxicity test was to expose a group of hamsters for 1 day (6 hr) to the highest practical concentration of respirable NEFA aerosol to investigate the resultant effects and thereby obtain guidelines for determining

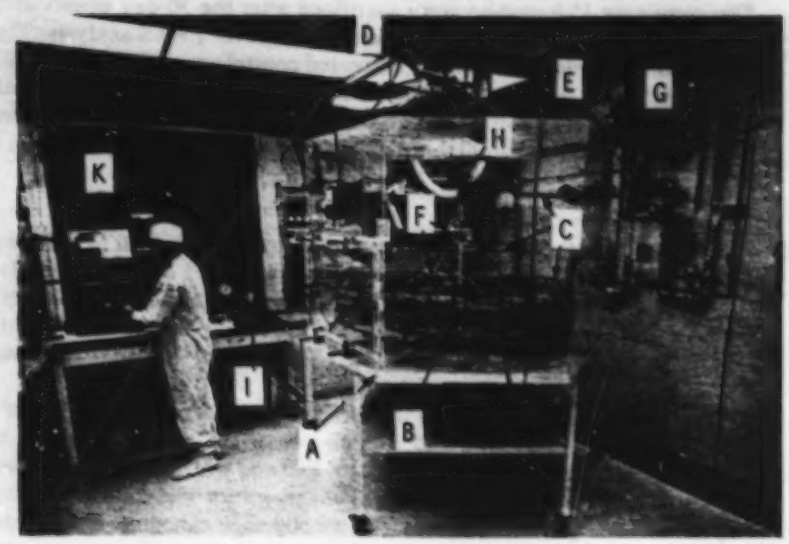

FIG. 1. Aerosol exposure system. A = Aerosol exposure chamber; B = hamster cages; C = drinking water system; D = Wright dust feed mechanism (WDFM); E = elutriator; F = aerosol sampling probes; G = air drier for providing dry air for aerosol generation by WDFM; H = mixing fan; I = press to pack fly ash into dust container of WDFM; K = microbalance for weighing filter paper samples.

suitable NEFA aerosol concentrations first for subchronic (2 months) and ultimately for life span exposures.

Seventeen 4-month-old male Syrian golden hamsters were exposed for 6 hr to NEFA aerosol. Mean total aerosol concentration was approximately 590 ± 21(SD) μg/liter; the "respirable" fraction was approximately 220 μg/liter. This high aerosol concentration was reached by operating two Wright dust feed mechanisms (WDFM; Wright, 1950) at near maximal speed. Total aerosol concentration was measured hourly for 4 hr by aspirating a known volume of air through a Gelman Metrical DM-450 membrane filter which was weighed before and after the sampling. The respirable fraction was measured continually using a portable battery-powered Casella type 113A gravimetric dust sampler which was suspended above the center of the animal cage in the exposure chamber. The instrument samples at a flow rate of 2.5 liter/min and selectively transmits particles which conform to the British Medical Research Council's definition of a "respirable" aerosol.

Seven days postexposure, two randomly selected hamsters were sacrificed to determine their NEFA lung burdens. Tissue samples were analyzed for nickel by flame atomic absorption spectrometry (FAAS) using the 232.0-nm nickel line with a bandpass of 0.2 nm and an oxidizing air/acetylene flame. Samples were prepared for analysis by digestion with concentrated nitric acid containing 0.5% hydrochloric acid and heating for 20 min at 400°C in a muffle furnace. Digestion and muffling were repeated until the tissue was completely ashed. The mineral residues were

then dissolved in 2 N nitric acid for analysis, using FAAS and analyzing for nickel. The remaining 15 hamsters were sacrificed after the 30-day observation period. The lungs of five of these animals were collected for FAAS analysis. The lungs of the remaining 10 animals and of 5 untreated controls were gently perfused through the trachea with 10% neutral buffered formalin to approximately normal volume, followed by tracheal ligation. After fixation, the lungs were embedded in paraffin, stained with hematoxylin and eosin, and sectioned for histopathologic examination.

Subchronic Toxicity Test

Sixty 6.5-month-old male hamsters were randomly divided into three groups of 20 hamsters each. Group 1 was exposed for 6 hr/day, 5 days/week for 2 months to a respirable NEFA aerosol concentration intended to be approximately 200 μg/liter. Group 2, serving as fly ash (FA) control group, was treated identically but was exposed to FA aerosol rather than NEFA aerosol. Group 3, otherwise treated identically, served as untreated control group.

The animals were maintained in individual 150-cm^2 compartments of stainless-steel wire mesh cages and had free access to Wayne Lab-Blox F4 laboratory animal feed (Allied Mills, Inc., Chicago, Ill.) and water. The animals remained in their cages in the 2700-liter exposure chambers for the duration of the 2-month experiment except for brief periods of weekly cage changing, and weighing of the animals.

The dust preparation prior to packing of the dust into the cups of the WDFM

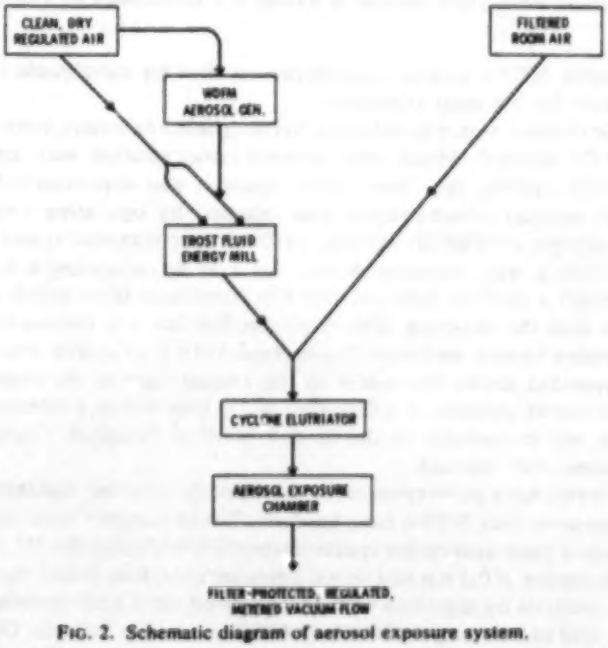

FIG. 2. Schematic diagram of aerosol exposure system.

consisted of sieving using a 212-μm sieve, and heated (100°C) vacuum desiccation.

To increase the respirable fraction of the aerosol after it had been generated by the WDFM, a small fluid energy mill (Trost Gem T Research Model Jet Mill, Garlock, Inc., Plastomer Products Div., Trost Air Mill Department, Newtown, Pa.) was placed downstream of the WDFM (Fig. 2) (Moss and Milliman, 1978). The Trost mill takes dust, fed by gravity or airstream, and entrains it into two opposing, turbulent jets. The particles collide with one another and break up until small enough to escape the impaction chamber. A cyclone elutriator, positioned between the Trost mill and the exposure chamber, removed most of the particles with aerodynamic diameters larger than 10 μm.

Total aerosol concentration was measured as in the acute toxicity test, namely, by aspirating a known volume of air through each of two Gelman Metrical DM-450 membrane filters which were weighed before and after the measurements. The filters were positioned opposite one another, approximately 45 cm centripetally from the chamber walls, and about 45 cm above the cages. Three 15-min samples were taken during each 6-hr exposure period, namely, 30 min after starting the exposures, after 3 hr, and before ending the exposures. The respirable fraction was measured simultaneously with the total aerosol concentration measurements, but for 20 min each, using the Casella gravimetric dust sampler. Particle size distribution was determined using an Andersen cascade impactor (Andersen, 1966).

The mean respirable aerosol concentration for the 40 exposure days was 183 ± 27 μg/liter (SD) in the FA exposure chamber compared to 188 ± 49 μg/liter in the NEFA chamber. The mass median aerodynamic diameters were 3.1 (GSD 2.0) and 2.8 μm (GSD 2.1), respectively. The air flow rate through the chambers ranged from 3 to 4 air changes/hr. Both the NEFA and the FA aerosol particles consisted of solid spheres and small carbon flakes (Figs. 3 and 4).

Starting 64 hr after completion of the 2-month exposures, all surviving animals were sacrificed by intraperitoneal injection of sodium pentothal and exsanguination. Lungs with the trachea were then removed and the carcass discarded. The lungs of half the animals from each group were prepared for histopathologic examination as described. The remaining lungs were analyzed for nickel to determine the quantity of NEFA retained in the lungs, using FAAS.

RESULTS

Acute Toxicity Test

Histopathologic changes in the lungs of the hamsters, exposed for 6 hr to a mean respirable NEFA aerosol concentration of 220 μg/liter, are summarized in Table I. A very small to small accumulation of dark brown granular pigment, resembling NEFA, was present in the lungs of the majority of the NEFA-treated hamsters. This pigment was primarily phagocytosed in macrophages located in areas of bronchiolization of alveolar epithelium. The pigment was also occasionally present in the interstitial areas. Bronchiolization of the alveolar epithelium was present in both the NEFA-treated hamsters and controls, but appeared increased in the NEFA-treated hamsters. No other treatment-related changes were observed.

FAAS analysis of the lungs from the two hamsters sacrificed 1 week after the acute 6-hr exposure showed an average of 7 μg nickel per lung, indicating a

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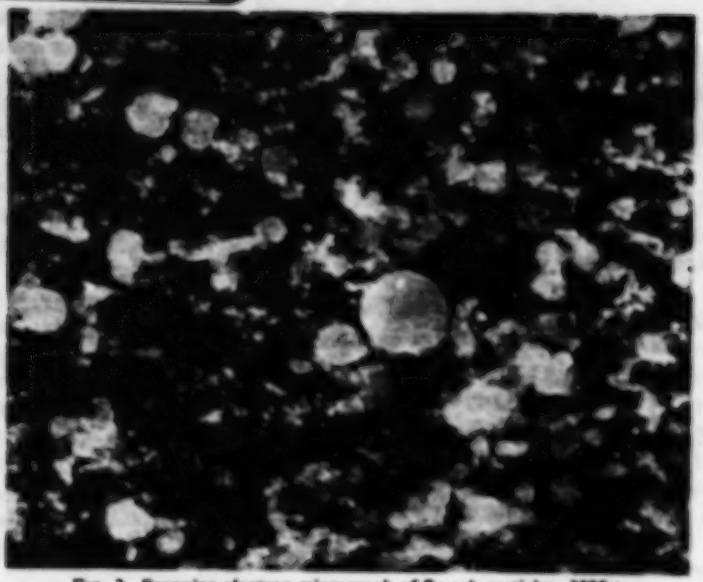

FIG. 3. Scanning electron micrograph of fly ash particles, 3000×.

FIG. 4. Scanning electron micrograph of fly ash particles, 10,000×.

TABLE I
SUMMARY OF HISTOLOGIC LUNG CHANGES IN HAMSTERS AFTER ONE 6-hr EXPOSURE TO NEFA AEROSOL[a]

Exposure group and animal no.	Histologic changes in lungs[b]	
	Bronchiolization	Pigment
Control		
1	0	0
2	0	0
3	1	0
4	0	0
5	1	0
NEFA		
2	1	1
4	1	1
6	0	0
7	1	1
8	0	0
11	2	2
13	1	1
14	2	2
15	1	1
16	1	1

[a] Respirable concentration 220 μg/liter.
[b] Key: 0 = Normal; 1 = very slight (or very small amount); 2 = slight (or small amount); 3 = moderate; 4 = marked; 5 = extreme.

pulmonary deposition and retention of about 78 μg NEFA. The lungs of the five hamsters sacrificed 30 days after exposure contained an average of 6.8 μg nickel per lung, or 75 μg NEFA. These calculations are based on the assumption that the nickel in the NEFA is retained and does not leach appreciably from the NEFA in tissue fluids. This assumption is supported by the observation that the average nickel content per lung remained practically the same from 7 days postexposure (7 μg) to 30 days postexposure (6.8 μg) which would not be the case if the nickel were to leach from the NEFA.

Subchronic Toxicity Test

The group mean body weights (MBW) as a function of time and treatment for the NEFA, the FA, and the control animals are shown in Fig. 5. The preexposure MBW of the FA group was higher and that of the NEFA group was lower than that of the controls. This difference was maintained to varying degrees throughout the 2-month exposure. A repeated-measures analysis of the weight data using a least significant difference multiple comparison procedure shows that the FA animals were significantly heavier ($P < 0.05$) than the other groups. Since this group was significantly ($P < 0.05$) heavier than the others at the first (preexposure) measurement and since the curves of MBW as a function of time are fairly parallel, this difference cannot be considered a treatment effect.

All three weight curves are dish shaped, showing a gradual initial decrease, followed by a weight-stable period and a final gradual increase toward the original

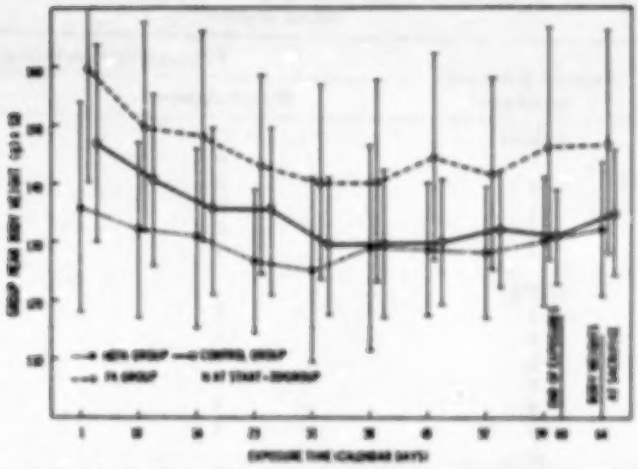

FIG. 5. Group mean body weights of male Syrian golden hamsters as a function of time and treatment.

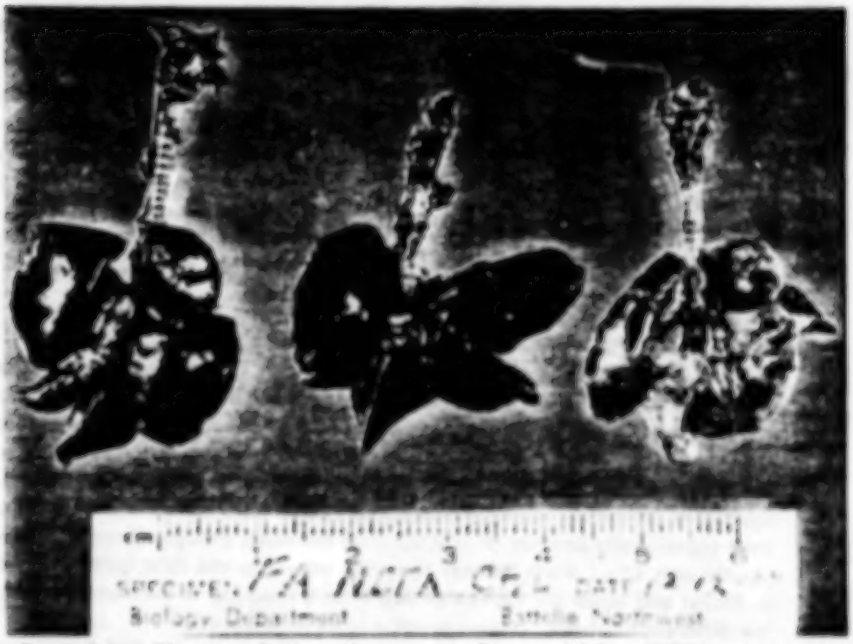

FIG. 6. Lungs of hamsters after 2-month exposures to FA aerosol (left) and NEFA aerosol (center). Lung of control animal is shown at right.

values. Stress responses often show a similar behavior. In our case, the dust exposures must be ruled out as a causative stress factor as the controls showed the same behavior. Perhaps the stress of transferring the animals from plastic group cages to the individual wire mesh cages 3 days prior to the start of the exposures accounts for this phenomenon.

During the 2-month exposure period four control animals and one NEFA-exposed animal died of undetermined but apparently not treatment-related causes. The NEFA-exposed animal had developed ascites. Its girth increased considerably and its death weight was 188 g. At necropsy, 7 to 8 ml of fluid was found in the abdominal cavity. The surviving animals showed no apparent ill effects.

At necropsy after 2 months of exposure, the lungs of the FA- and NEFA-exposed hamsters were black or dark brown in contrast to the normal pink color of the lungs from the control hamsters (Fig. 6). Results of the histopathologic examination are summarized in Table 2. Black or dark brown granular dust was scattered in alveoli throughout the lung sections but appeared somewhat more concentrated in subpleural and peribronchiolar areas (Fig. 7). The dust was generally phagocytosed by alveolar macrophages with the macrophages so heavily laden with dust that normal cellular details were obscured (Fig. 8). Aside from the increased number of macrophages with phagocytosed dust, there was minimal inflammatory reaction. There was slight thickening of alveolar septa and some bronchiolization of alveolar epithelium. No differences between the FA- and NEFA-exposed groups were observed.

Weights of the wet (fresh) lungs analyzed for nickel and the results of the FAAS analyses are shown in Table 3. One-way analysis of variance of the wet lung weights shows that the means of the three groups are not equal. Scheffe's S method was used to make all possible comparisons among means. Results of these comparisons show that the FA and NEFA mean wet lung weights are not significantly different from one another but are significantly ($P < 0.001$) higher than that of the controls. An average of approximately 510 μg nickel per lung was found in the hamsters after 2 months of exposure to NEFA at a mean respirable aerosol concentration of about 190 μg/liter. Quantities of nickel in the FA-exposed animals and in the controls were below the detection limit of the method, i.e., <1 μg. Based on a 9% w/w nickel content of the NEFA, and assuming that there was no appreciable leaching of nickel from the NEFA, the average quantity of NEFA retained per lung in the NEFA-exposed hamsters after 2 months of exposures (40 six-hour exposures) was approximately 5.7 mg.

DISCUSSION

Neither the acute nor the subchronic exposures had any noticeable effect on the well-being, body weight, or life span of the exposed animals although the respirable fractions of the aerosol concentrations were very high, namely, 220 and 183 μg/liter, respectively.

Substantial quantities of NEFA were inhaled and retained in the lungs as evidenced by the dark color of the lungs at necropsy (Fig. 6), the histopathologic findings (Table 2, Fig. 7), and quantitatively by the FAAS analyses (Table 3). None of the trace elements naturally occurring in FA were present at levels

TABLE 3
SUMMARY OF LUNG CHANGES AFTER 2 MONTHS OF EXPOSURE TO NEFA AND FA

Exposure group and animal no.	Lung changes*			
	Dust	Interstitial reaction	Bronchiolization	Miscellaneous
NEFA				
1		3	2	1
3		3	2	
5		3	2	1
7		3	4	Atelectasis (died)
7		3	2	
9		4	3	Very slight emphysema and pleuritis
11		3	2	
12		3	2	
14		3	2	
15		3	2	
16		4	3	1
FA				
1		3	2	
2		3	2	
5		3	2	
5		3	2	
7		3	2	
8		3	2	
10		3	2	
12		3	2	1 Bronchiolar +dil
14		4	3	
15		3	2	
Controls				
1			1	1
3	N			
5		1		
9	N			
11				2
13				Slight leukocytosis
15	N			
16				1
17			1	2

* Key: N = Normal; 1 = very slight (or very small amount); 2 = slight (or small amount); 3 = moderate; 4 = marked; 5 = extreme.

sufficiently high to permit FAAS analysis. While no quantitative data are available for the amount of FA present in the lungs of the FA-exposed animals, it is reasonable to assume that the quantities of FA retained in the lungs were very similar to those of NEFA as determined by FAAS. This statement is based on the observations that (a) NEFA and FA particles have very similar particle size characteristics and can thus be assumed to have very similar aerodynamic (and thus pulmonary deposition and retention) characteristics; (b) at necropsy, the lungs of the

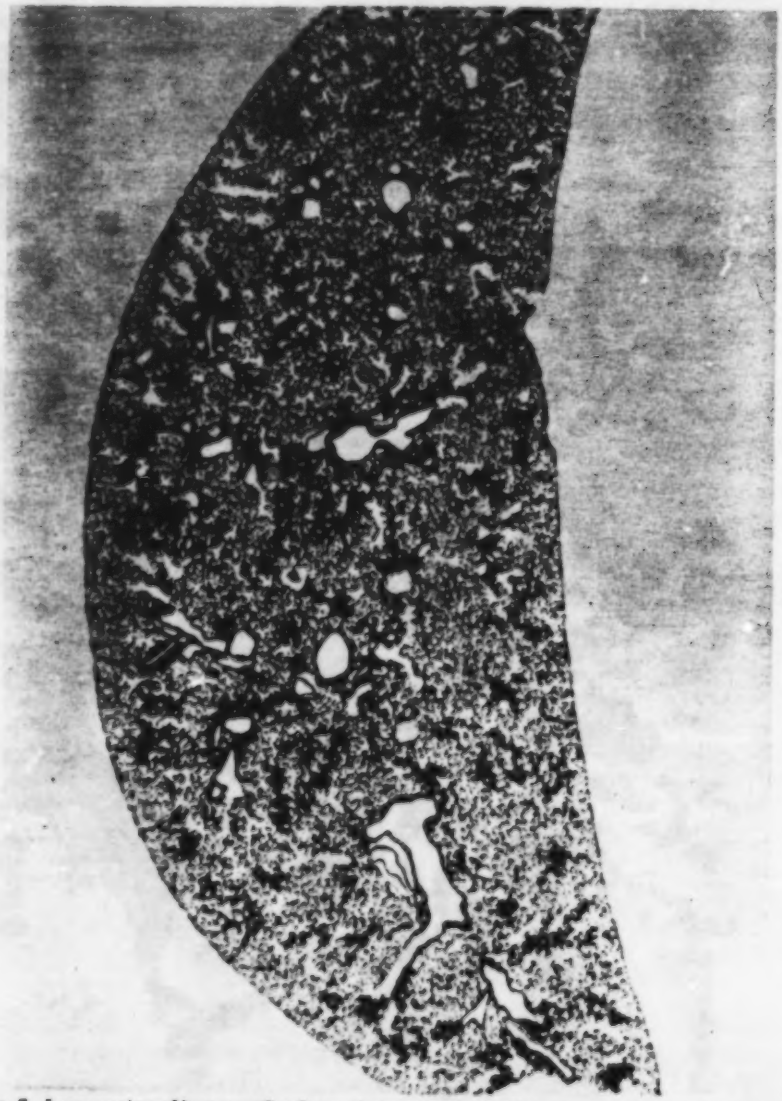

FIG. 7. Lung section of hamster after 2-month exposure to NEFA aerosol, showing dark, granular dust scattered in alveoli throughout the lung with local concentrations in subpleural and peribronchiolar areas. H&E, 12.5×.

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FIG. 8. Lung section, similar to the one shown in Fig. 7, at higher magnification, showing many dust-laden alveolar macrophages. H&E, 500×.

TABLE 3

BODY WEIGHTS, WET LUNG WEIGHTS, AND QUANTITIES OF NICKEL FOUND BY FLAME ATOMIC ABSORPTION SPECTROSCOPY IN THE LUNGS OF HAMSTERS AFTER 2 MONTHS OF EXPOSURE[a]

Exposure group	N	Mean body weight (g ± SD)	Mean wet lung weight (g ± SD)	Mean Ni in lung (μg ± SD)	Equivalent mg NEFA[b] (mean ± SD)
NEFA	9	133 ± 12	1.466 ± 0.120**	511 ± 70	5.67 ± 0.77
FA	10	138 ± 17	1.510 ± 0.143**	<1	—
Controls	8	133 ± 10	0.930 ± 0.101	<1	—

[a] Limit of detection ~1 μg.

[b] Calculated assuming 9% w/w Ni in Ni-enriched fly ash (NEFA).

** Significantly different from controls, $P < 0.001$.

NEFA- and of the FA-exposed animals looked equally dark; and (c) histopathologically, the grading of dust found in the lungs of the NEFA- and of the FA-exposed animals was very similar (Table 2).

There was no apparent difference between the effects of NEFA and those of FA under the investigated conditions of acute and subchronic exposures. Whether or not this holds true also for chronic exposures is presently being investigated (see below).

The pulmonary retention of substantial quantities of NEFA and FA dust notwithstanding, histopathologic examination revealed only a nonspecific dust reaction even after 2 months of exposure. This indicates a rather low acute and subchronic toxicity of both NEFA and FA in the hamster lung. Similar findings were observed in our laboratory when hamsters were exposed to NiO (Wehner *et al.*, 1972, 1975) 6 hr/day, 5 days/week, for 3 weeks (mean respirable NiO aerosol concentration ~40 μg/liter); for 3 months (~60 μg/liter); and for life (53 μg/liter). None of the animals died during the 3-week and the 3-month exposures. The animals on life span exposures that died during the first few months from causes unrelated to the exposures had heavy burdens of NiO but showed relatively little cellular response. Both proliferative and inflammatory cellular response increased only later in the life span experiment, leading eventually to severe pneumoconiosis with severe degrees of lung consolidation. We found NiO to have low solubility in tissue fluids and relatively long residence time in the lung; 45 days postexposure, 45% of the NiO deposited in the lung was still present. By contrast, exposure of hamsters to CoO (mean respirable aerosol concentration ~110 μg/liter) 3 hr/day resulted in the death of all hamsters within 4 days (Wehner *et al.*, 1972). Within 7 days, essentially all the CoO had been cleared from the lungs of other hamsters exposed to CoO, while more than 70% of the NiO was still present at that time.

Based on a 9% w/w nickel content of the NEFA, and assuming that there was no appreciable leaching of nickel from the NEFA, the average quantity of NEFA retained in the lungs of the NEFA-exposed hamsters after 2 months of exposures (40 six-hour exposures) was approximately 5.7 mg. By comparison, one 6-hr exposure (acute toxicity test) showed an average of 7 μg nickel per lung, or 78 μg NEFA, in the two hamsters sacrificed 1 week after exposure, and 6.8 μg nickel per lung, or 75 μg NEFA, in the five hamsters sacrificed 30 days after exposure.

Thus, the 40 exposures of the subchronic toxicity test resulted in a 76-fold higher pulmonary NEFA retention than the one exposure of the acute toxicity test, i.e., almost twice as much nickel was found in the lungs of the subchronic exposure animals as might be expected based on the acute toxicity test, not considering any clearance mechanisms. The following explanations might be considered to account for this difference between observed and expected lung burden.

(1) The time from completion of the exposures to sacrifice (64 to 70 hr) was not long enough to allow for mucociliary clearance of nickel deposited in the ciliated part of the respiratory tract. The observed pulmonary NEFA burden of 5.7 mg would thus comprise the deep lung burden plus some quantity not yet cleared by mucociliary action. However, our experience in the past indicates that in hamsters mucociliary clearance is usually complete within 20 min or less, given a normally functioning clearance mechanism.

(2) Repeated 6-hr exposures to such high aerosol concentrations might have "overwhelmed" the clearance mechanism, making it less and less efficient, i.e., resulting in larger quantities of material retaincd.

(3) The difference of a factor of ~2 between observed and expected lung burdens falls within the inherent variations of such biological systems.

(4) The hamsters of the acute toxicity test were 4 months old; those of the subchronic toxicity test were almost 7 months old. However, their average body weights were similar, namely, 126 g for the former and from 133 to 138 g for the latter groups. This indicates that the 4-month-old group had reached adulthood and thus can be assumed to have respiratory function and pulmonary deposition/retention characteristics similar to those of the 7-month-old group.

Whatever the case may be, NEFA (and FA if it behaves similarly in the lung) appears to have a rather long biological half-life in the hamster lung as indicated by the observation that there was practically no difference in the quantities of NEFA in the lungs of the two hamsters sacrificed 1 week after the acute toxicity test (mean 78 μg) and those of the five hamsters sacrificed 30 days after exposure (mean 75 μg).

The percentage of inhaled NEFA that was deposited in the deep lung may be estimated from the following known parameters: respirable aerosol concentration, exposure time, minute volume of the hamster (60 ml; Gleysteen and Stroud, 1974), and the quantity of NEFA deposited in the lungs of the exposed animals. These calculations indicate that during the 6 hr of exposure to 220 μg/liter respirable NEFA aerosol the animals inhaled an average of approximately 4.75 mg NEFA of which approximately 80 μg, i.e., less than 2% of the inhaled quantity, was deposited in the deep lung.

The mean wet lung weights of both the FA- and the NEFA-exposed groups were significantly ($P < 0.001$) higher than that of the controls, namely, 1.51 and 1.47 g versus 0.93 g, respectively (Table 3). If the assumption is correct that the lung weight increase is due to early lung changes as a consequence of the NEFA and FA exposures, lung weights might be a more sensitive criterion for early changes than the histopathologic changes observed under the microscope.

To answer some of the questions raised by the findings described in this paper, we have begun chronic exposures of hamsters to two levels of NEFA aerosol (15

and 75 μg/liter) and to FA (75 μg/liter) which are planned for up to 20 months. In addition, we are planning to determine pulmonary deposition, translocation, and clearance characteristics of NEFA in the hamster, using neutron-activated NEFA and an experimental approach similar to that described for a study with neutron-activated talc (Wehner *et al.*, 1977).

In conclusion, it can be stated that the results of this study indicate low acute and subchronic toxicity of NEFA and FA, as well as slow lung clearance. These findings suggest low solubility of the substances in body fluids. Enrichment of fly ash with nickel did not enhance its biological effects in hamsters under the described conditions, but facilitated convenient determination of lung burdens. Wet lung weights might be a sensitive criterion for early lung changes.

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